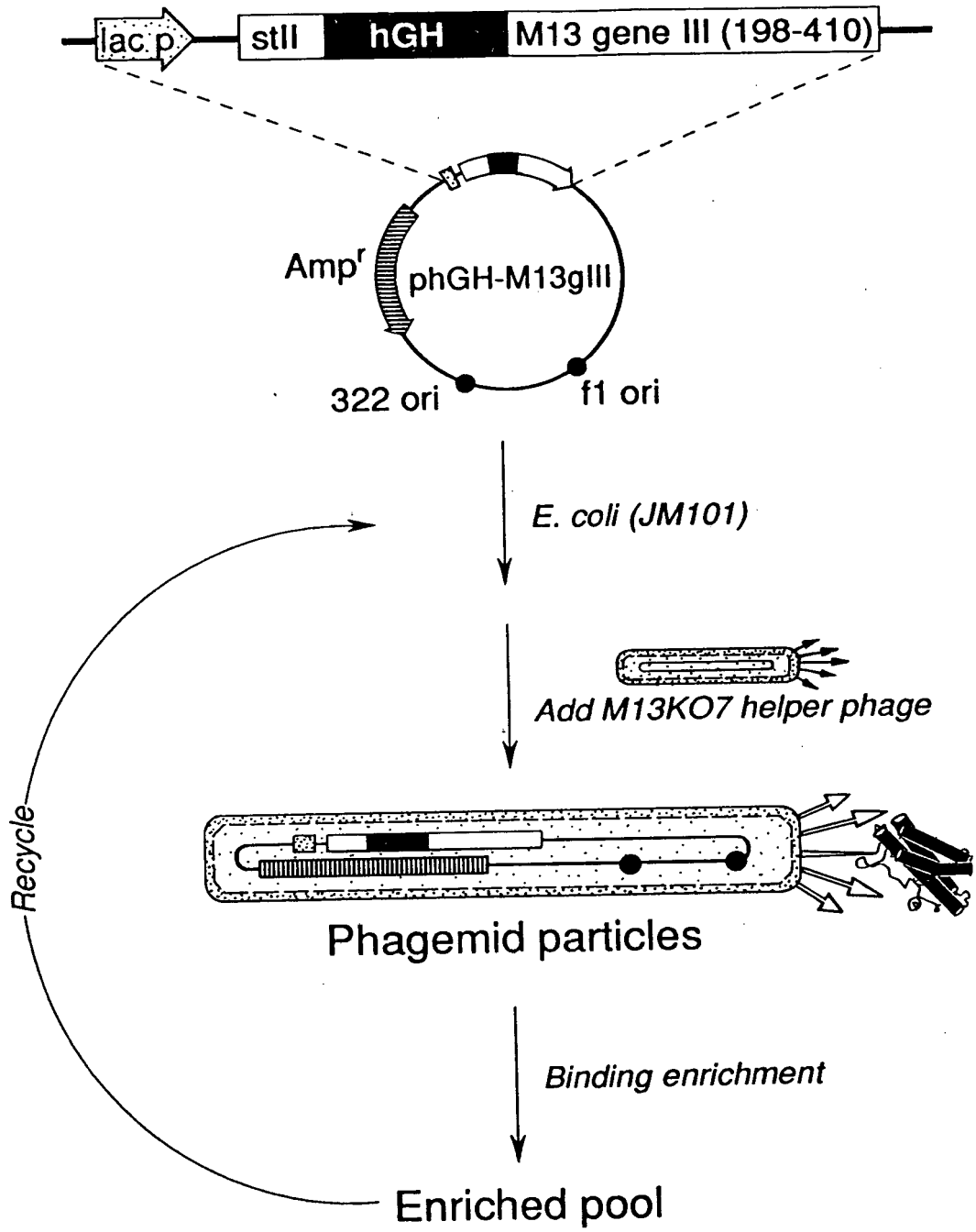

lac p
stII
hGH
M13 gene III (198-410)
Amp^r
phGH-M13gIII
322 ori
f1 ori
E. coli (JM101)
Add M13KO7 helper phage
Phagemid particles
Binding enrichment
Enriched pool
Recycle

FIG. 1

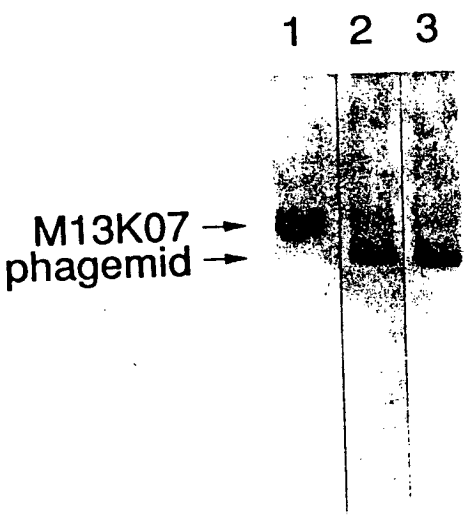


FIG. 2A

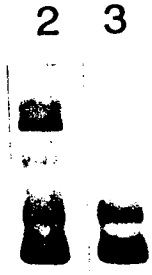


FIG. 2B

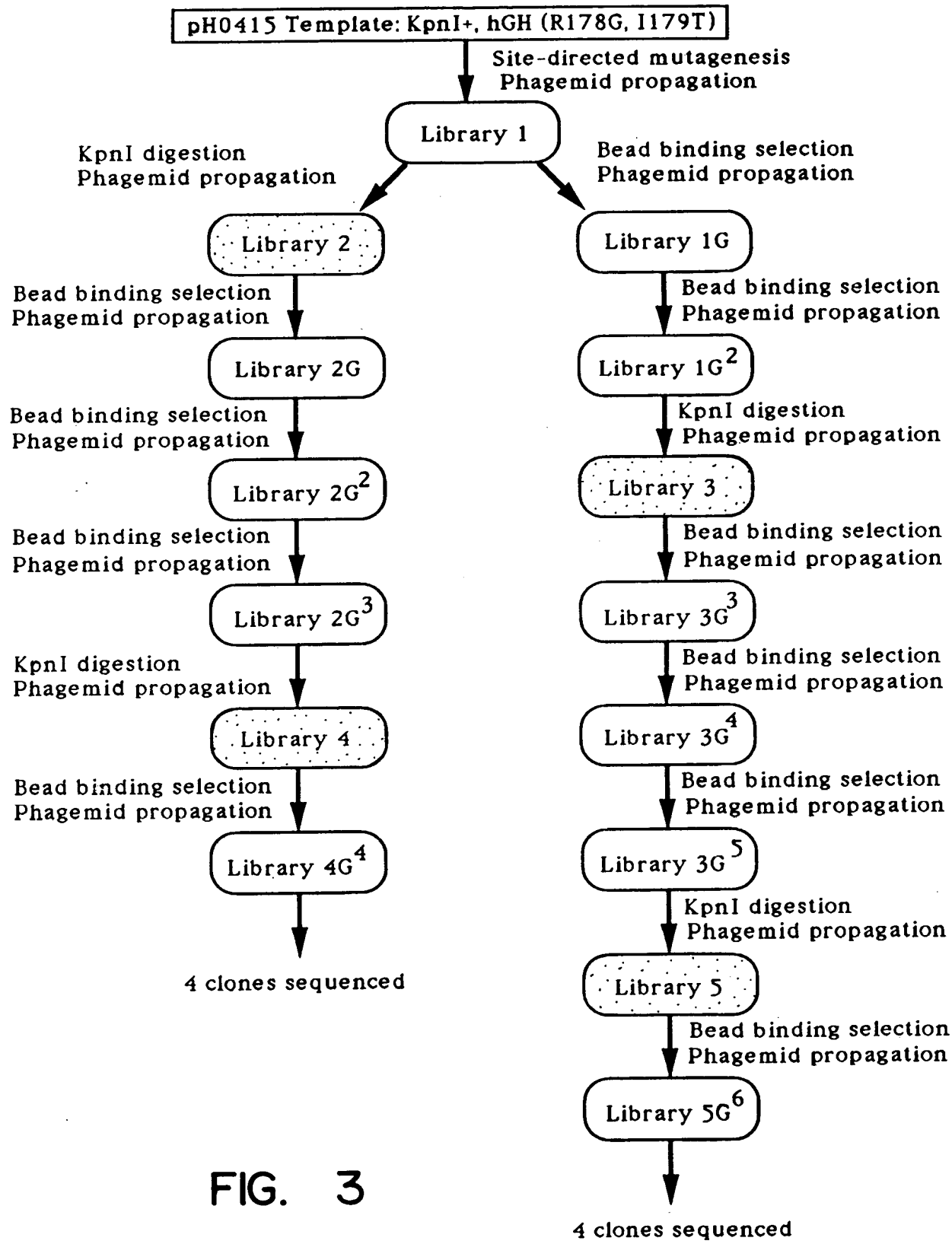

pH0415 Template: KpnI+, hGH (R178G, I179T)
Site-directed mutagenesis
Phagemid propagation
Library 1
KpnI digestion
Phagemid propagation
Bead binding selection
Phagemid propagation
Library 2
Library 1G
Bead binding selection
Phagemid propagation
Library 2G
Library 1G2
Bead binding selection
Phagemid propagation
KpnI digestion
Phagemid propagation
Library 2G2
Library 3
Bead binding selection
Phagemid propagation
Bead binding selection
Phagemid propagation
Library 2G3
Library 3G3
KpnI digestion
Phagemid propagation
Bead binding selection
Phagemid propagation
Library 4
Library 3G4
Bead binding selection
Phagemid propagation
Bead binding selection
Phagemid propagation
Library 4G4
Library 3G5
KpnI digestion
Phagemid propagation
Library 5
Bead binding selection
Phagemid propagation
Library 5G6
4 clones sequenced
4 clones sequenced

FIG. 3

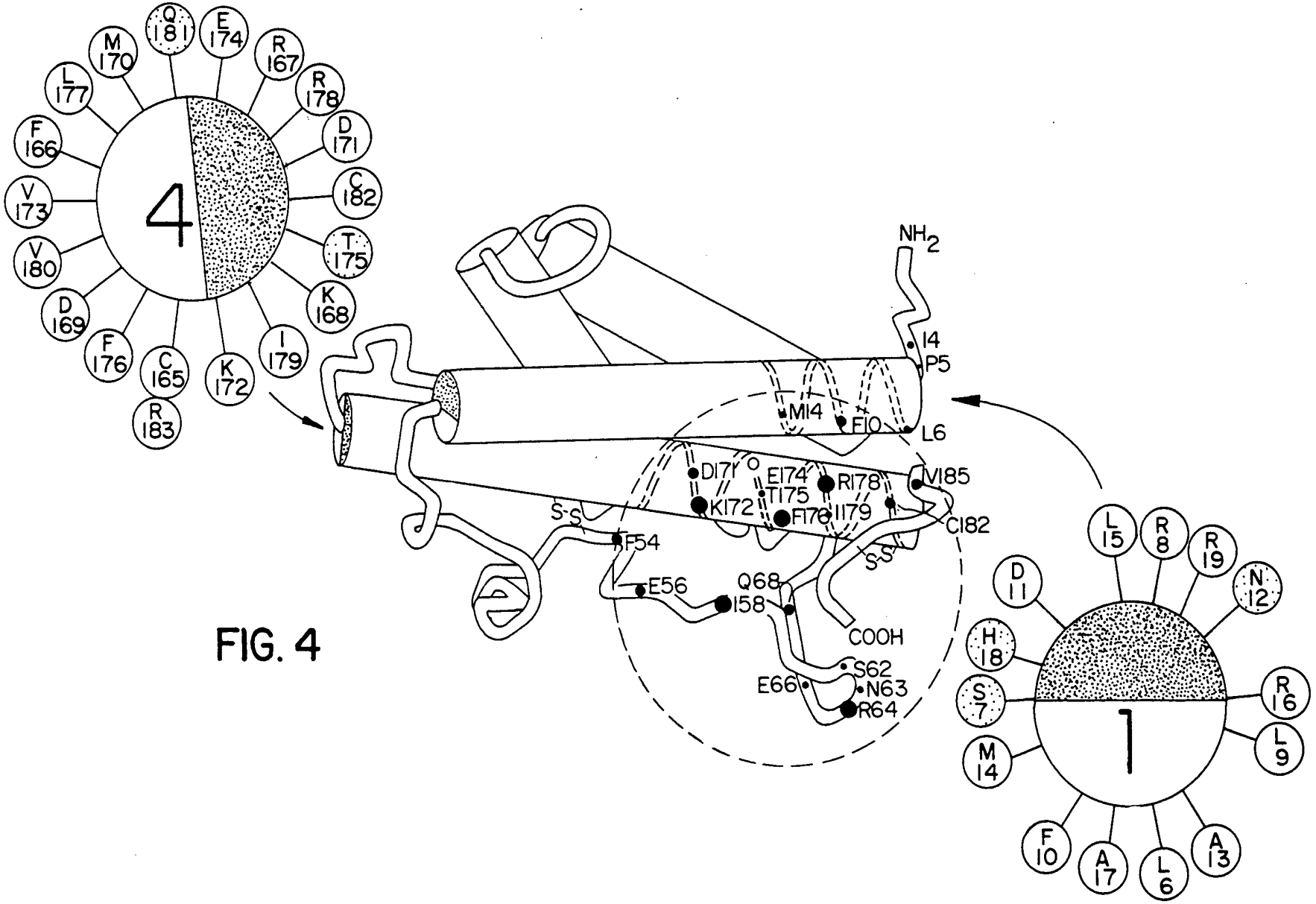


FIG. 4

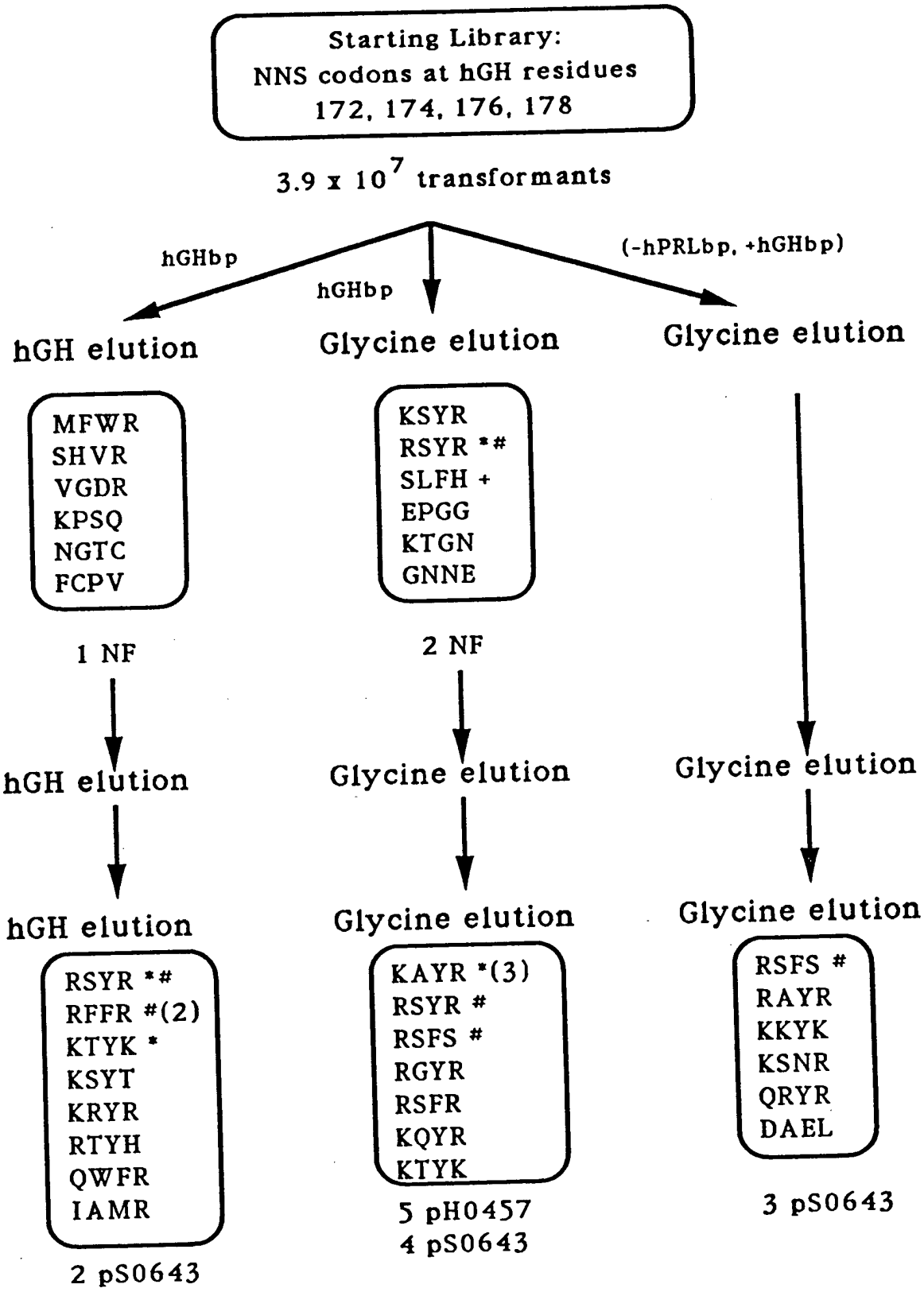

Starting Library:
NNS codons at hGH residues
172, 174, 176, 178
3.9 x 10^7 transformants
hGHbp
hGHbp
(-hPRLbp, +hGHbp)
hGH elution
Glycine elution
Glycine elution
MFWR
SHVR
VGDR
KPSQ
NGTC
FCPV
1 NF
KSYR
RSYR *#
SLFH +
EPGG
KTGN
GNNE
2 NF
hGH elution
Glycine elution
Glycine elution
hGH elution
Glycine elution
Glycine elution
RSYR *#
RFFR #(2)
KTYK *
KSYT
KRYR
RTYH
QWFR
IAMR
2 pS0643
KAYR *(3)
RSYR #
RSFS #
RGYR
RSFR
KQYR
KTYK
5 pH0457
4 pS0643
RSFS #
RAYR
KKYK
KSNR
QRYR
DAEL
3 pS0643

FIG. 5

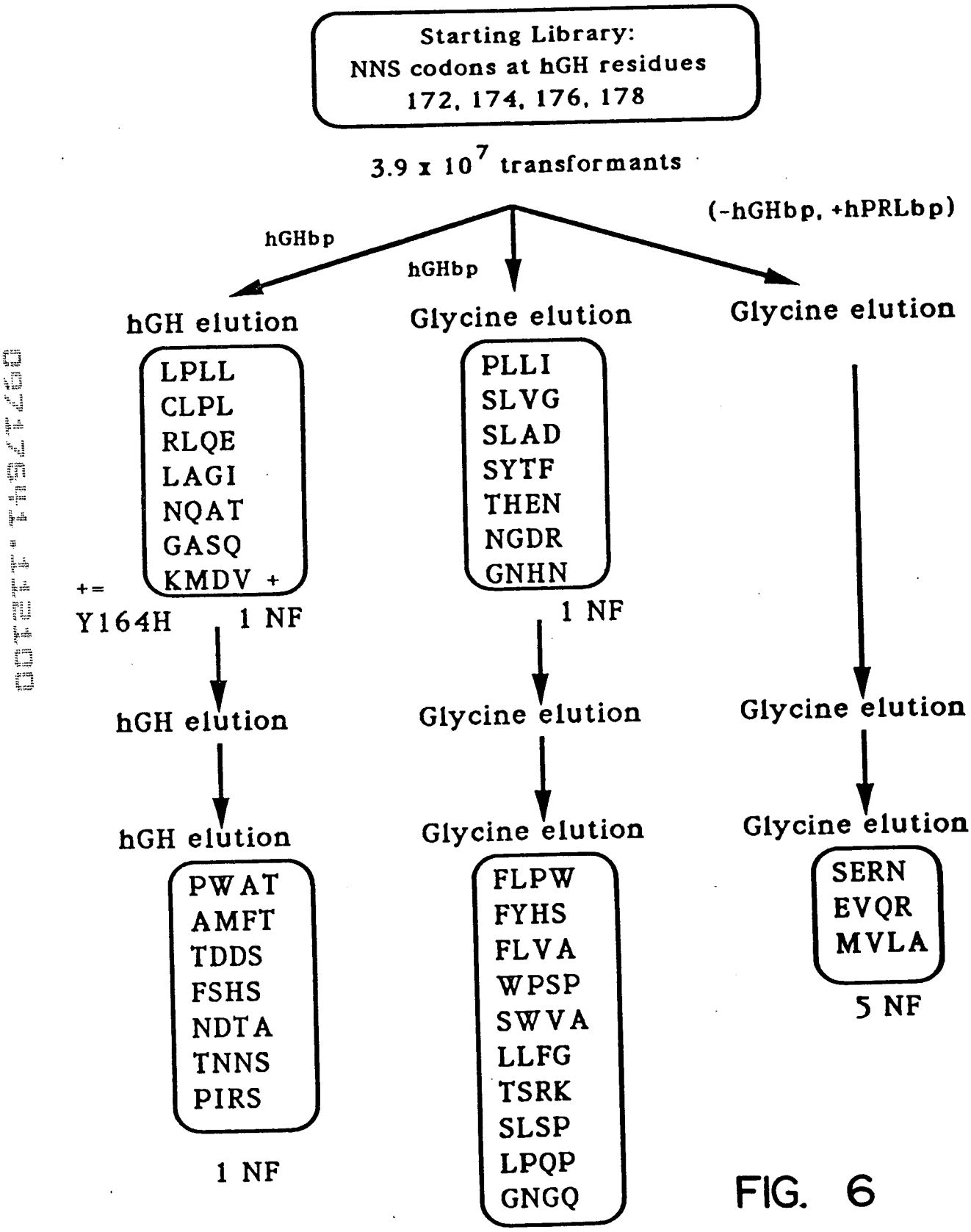

Starting Library:
NNS codons at hGH residues
172, 174, 176, 178
3.9 x 10^7 transformants
(-hGHbp, +hPRLbp)
hGHbp
hGHbp
hGH elution
Glycine elution
Glycine elution
LPLL
CLPL
RLQE
LAGI
NQAT
GASQ
KMDV +
+=
Y164H
1 NF
PLLI
SLVG
SLAD
SYTF
THEN
NGDR
GNHN
1 NF
hGH elution
Glycine elution
Glycine elution
hGH elution
Glycine elution
Glycine elution
PWAT
AMFT
TDDS
FSHS
NDTA
TNNS
PIRS
1 NF
FLPW
FYHS
FLVA
WPSP
SWVA
LLFG
TSRK
SLSP
LPQP
GNGQ
SERN
EVQR
MVLA
5 NF

FIG. 6

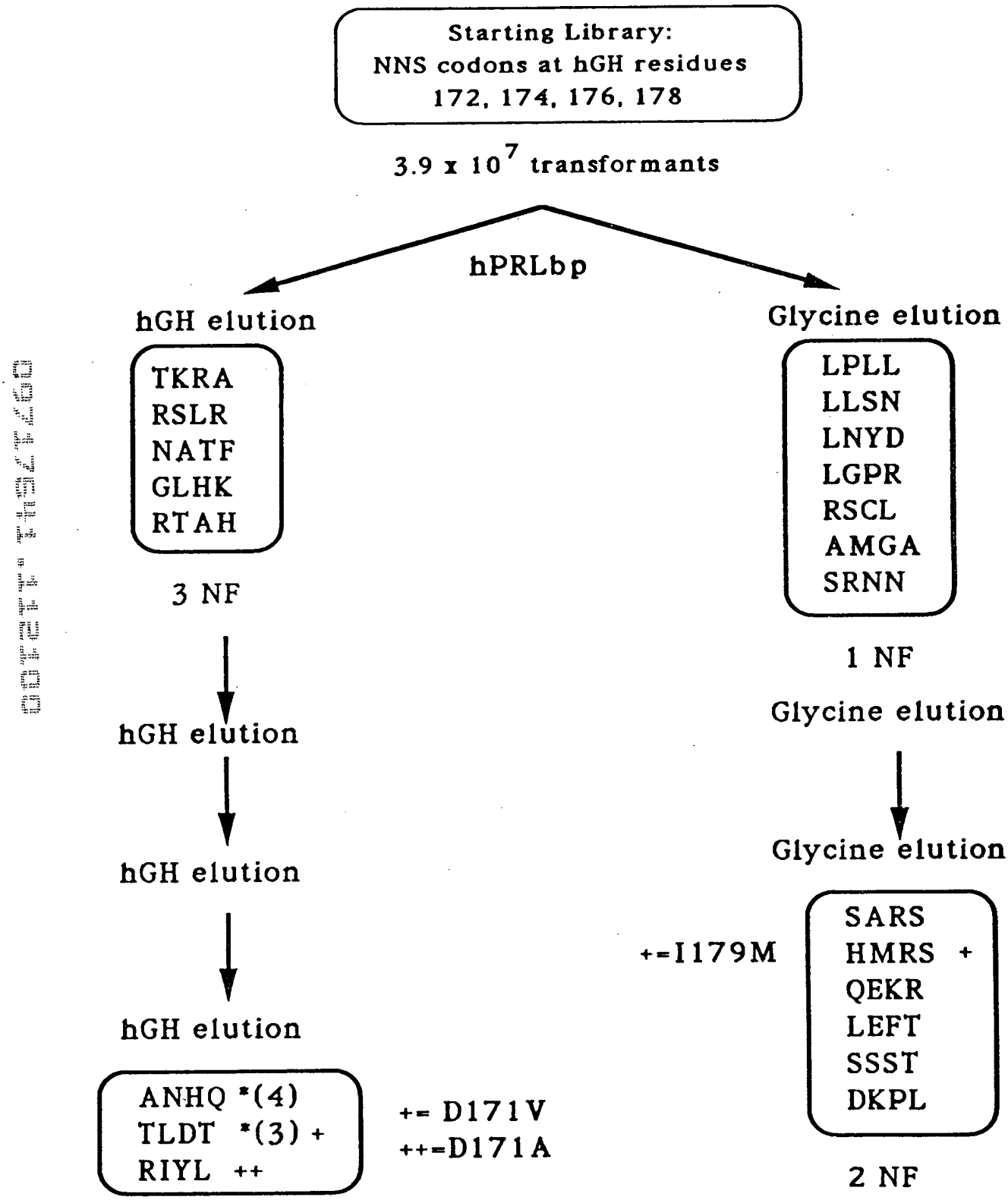

Starting Library:
NNS codons at hGH residues
172, 174, 176, 178
3.9 x 10^7 transformants
hPRLbp
hGH elution
Glycine elution
TKRA
RSLR
NATF
GLHK
RTAH
3 NF
LPLL
LLSN
LNYD
LGPR
RSCL
AMGA
SRNN
1 NF
hGH elution
Glycine elution
hGH elution
Glycine elution
hGH elution
SARS
HMRS +
QEKR
LEFT
SSST
DKPL
+=I179M
ANHQ *(4)
TLDT *(3) +
RIYL ++
+= D171V
++=D171A
2 NF

FIG. 7

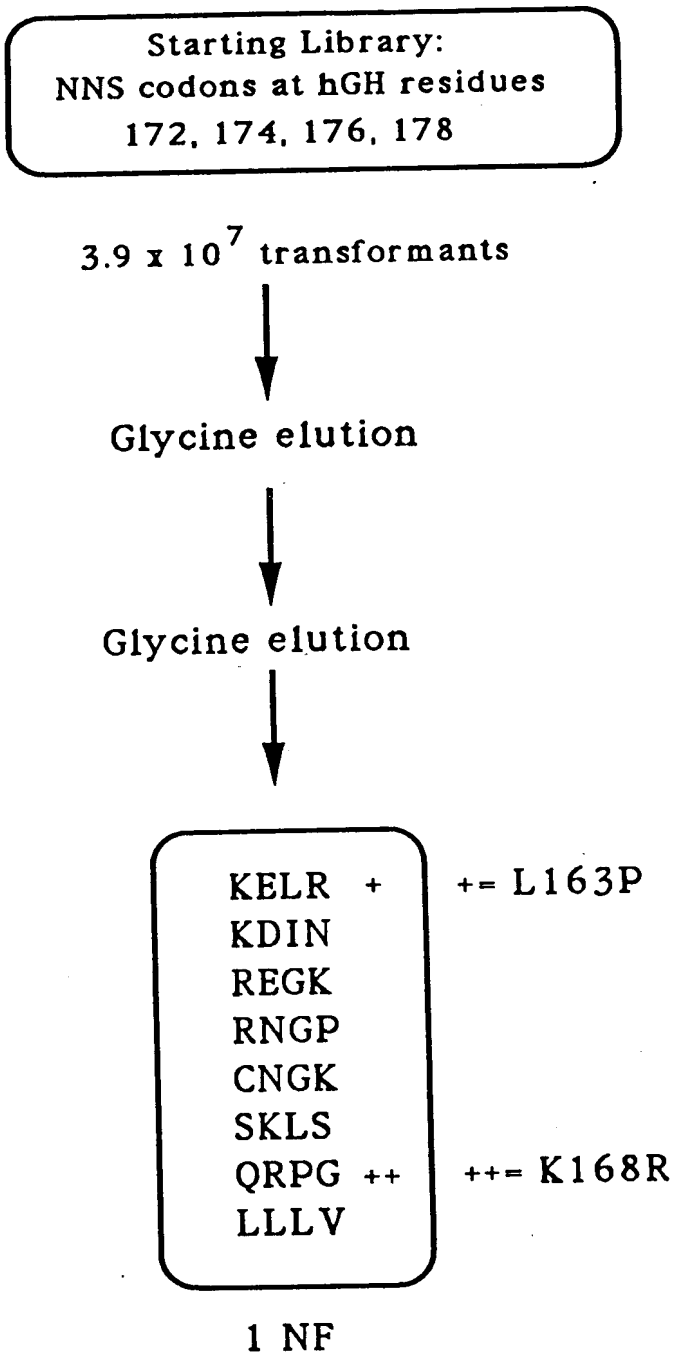
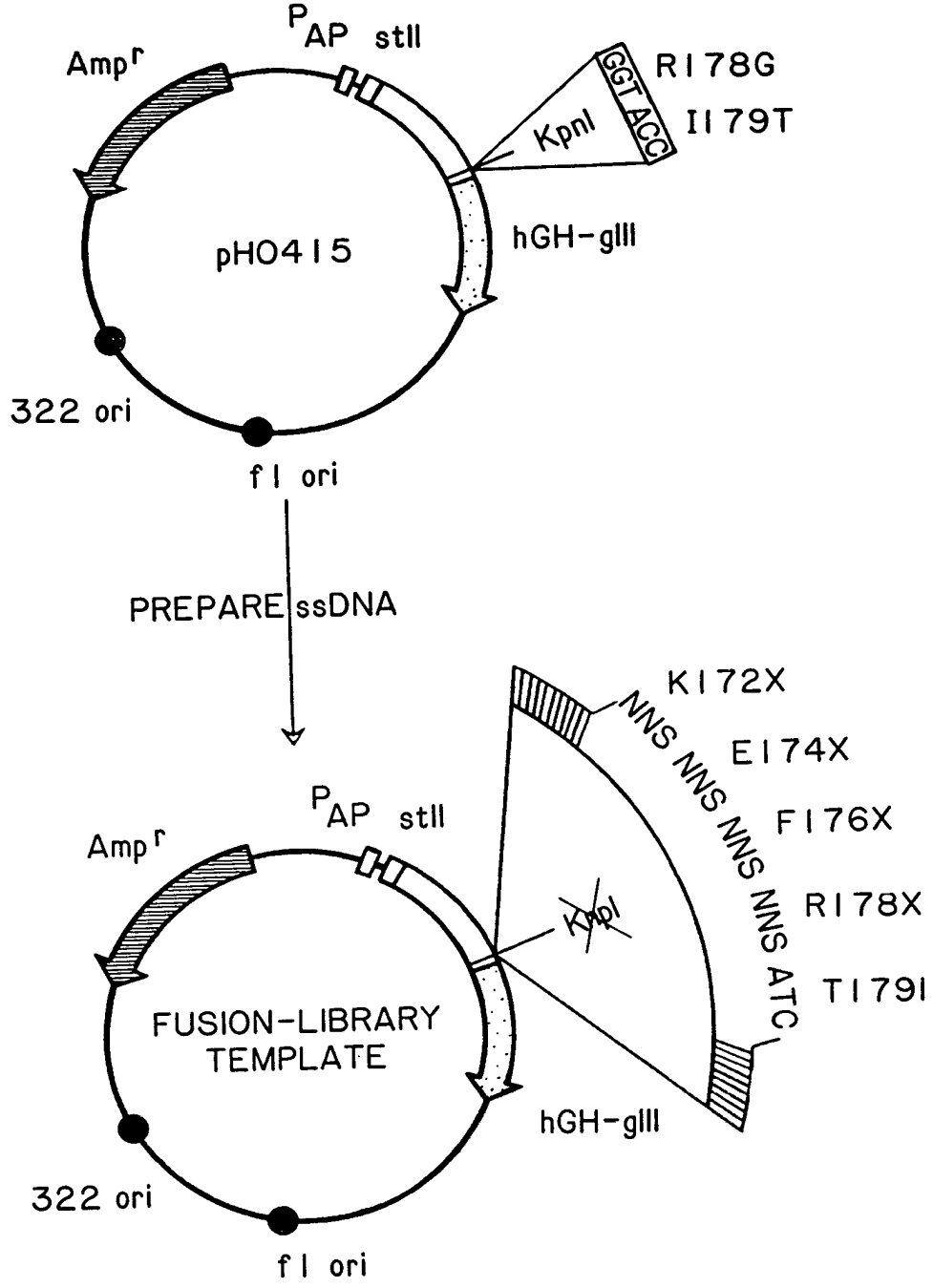

Starting Library:
NNS codons at hGH residues
172, 174, 176, 178
3.9 x 10^7 transformants
Glycine elution
Glycine elution
KELR +
KDIN
REGK
RNGP
CNGK
SKLS
QRPG ++
LLLV
+= L163P
++= K168R
1 NF

FIG. 8



THEORETICAL		EXPERIMENTAL
1.05×10^6	DNA seq.'s	8.2×10^6
1.6×10^5	A.A. seq.'s	??

FIG. 9

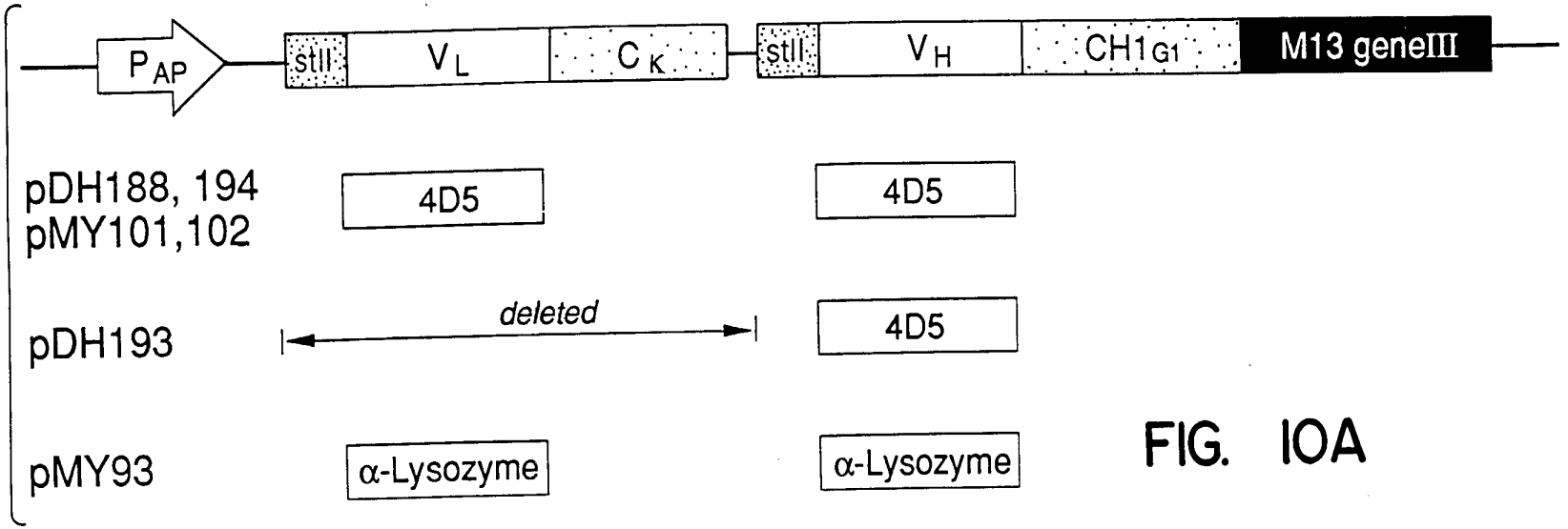


FIG. 10A

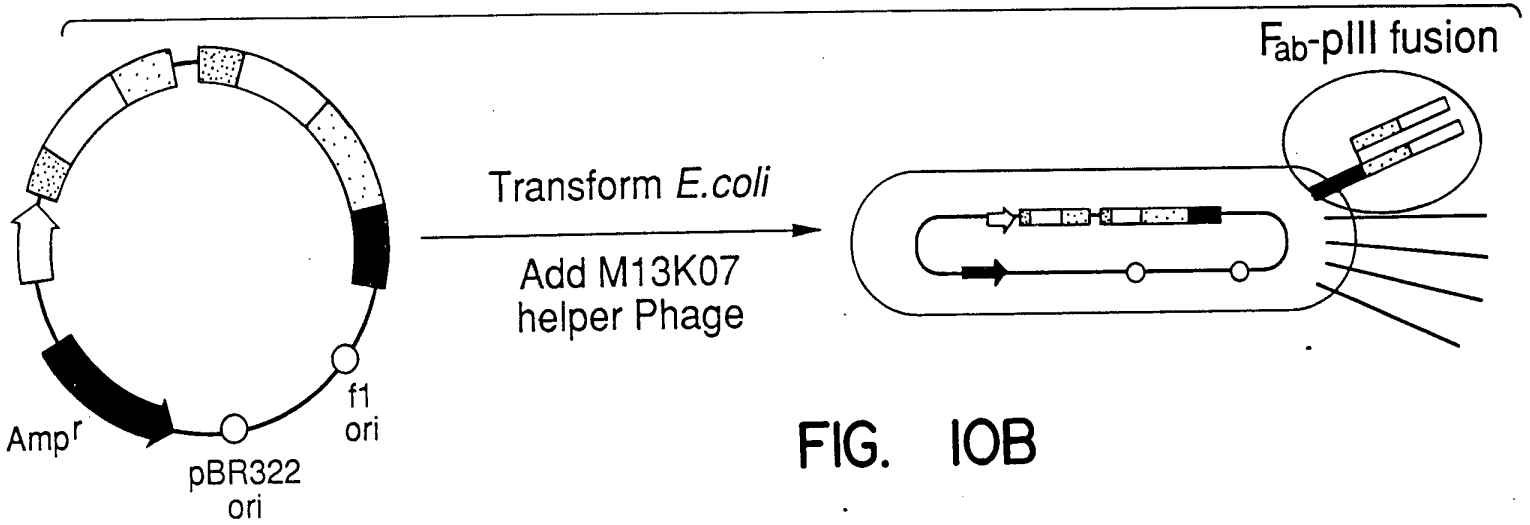


FIG. 10B

```
ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG  36
Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met
     1              5                  10

TTC GTT TTT TCT ATT GCT ACA AAC GCG TAC GCT GAT ATC  75
Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala Asp Ile
         15                  20                  25

CAG ATG ACC CAG TCC CCG AGC TCC CTG TCC GCC TCT GTG  114
Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
                 30                  35

GGC GAT AGG GTC ACC ATC ACC TGC CGT GCC AGT CAG GAT  153
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp
     40                  45                  50

GTG AAT ACT GCT GTA GCC TGG TAT CAA CAG AAA CCA GGA  192
Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
             55                  60

AAA GCT CCG AAA CTA CTG ATT TAC TCG GCA TCC TTC CTC  231
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu
 65                  70                  75

TAC TCT GGA GTC CCT TCT CGC TTC TCT GGA TCC AGA TCT  270
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser
         80                  85                  90
```

FIG. 11A

```
GGG ACG GAT TTC ACT CTG ACC ATC AGC AGT CTG CAG CCG 309
Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
                 95                 100

GAA GAC TTC GCA ACT TAT TAC TGT CAG CAA CAT TAT ACT 348
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr
    105                 110                 115

ACT CCT CCC ACG TTC GGA CAG GGT ACC AAG GTG GAG ATC 387
Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            120                 125

AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG 426
Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130                 135                 140

CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT 465
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val
        145                 150                 155

GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA 504
Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
                160                 165

GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC 543
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
    170                 175                 180
```

FIG. 11B

```
TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC 582
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
            185                 190

ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA 621
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195                 200                 205

GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC 660
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr
        210                 215                 220

CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC 699
His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
                225                 230

AGG GGA GAG TGT TAAGCTGAT CCTCTACGCC GGACGCATCG 740
Arg Gly Glu Cys
    235     237

TGGCCCTAGT ACGCAAGTTC ACGTAAAAAG GGTATCTAGA GGTTGAGGTG 790


ATTTT    ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT 828
         Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser
         238     240                 245
```

FIG. 11C

```
ATG TTC GTT TTT TCT ATT GCT ACA AAC GCG TAC GCT GAG 867
Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala Glu
    250                 255                 260

GTT CAG CTG GTG GAG TCT GGC GGT GGC CTG GTG CAG CCA 906
Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
            265                 270

GGG GGC TCA CTC CGT TTG TCC TGT GCA GCT TCT GGC TTC 945
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
275                 280                 285

AAC ATT AAA GAC ACC TAT ATA CAC TGG GTG CGT CAG GCC 984
Asn Ile Lys Asp Thr Tyr Ile His Trp Val Arg Gln Ala
        290                 295                 300

CCG GGT AAG GGC CTG GAA TGG GTT GCA AGG ATT TAT CCT 1023
Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Tyr Pro
                305                 310

ACG AAT GGT TAT ACT AGA TAT GCC GAT AGC GTC AAG GGC 1062
Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val Lys Gly
    315                 320                 325

CGT TTC ACT ATA AGC GCA GAC ACA TCC AAA AAC ACA GCC 1101
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala
            330                 335
```

FIG. 11D

```
TAC CTG CAG ATG AAC AGC CTG CGT GCT GAG GAC ACT GCC 1140
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
340                 345                 350

GTC TAT TAT TGT TCT AGA TGG GGA GGG GAC GGC TTC TAT 1179
Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
        355                 360                 365

GCT ATG GAC TAC TGG GGT CAA GGA ACC CTG GTC ACC GTC 1218
Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
                370                 375

TCC TCG GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG 1257
Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
    380                 385                 390

GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC 1296
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
            395                 400

CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG 1335
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
405                 410                 415

ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG 1374
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
        420                 425                 430
```

FIG. 11E

```
CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC 1413
His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                435                     440

TCC CTC AGC AGC GTG GTG ACT GTG CCC TCT AGC AGC TTG 1452
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
    445                     450                 455

GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC 1491
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
            460                     465

AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA TCT 1530
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
470                     475                     480

TGT GAC AAA ACT CAC ACA GGG CCC TTC GTT TGT GAA TAT 1569
Cys Asp Lys Thr His Thr Gly Pro Phe Val Cys Glu Tyr
        485                     490                 495

CAA GGC CAA TCG TCT GAC CTG CCT CAA CCT CCT GTC AAT 1608
Gln Gly Gln Ser Ser Asp Leu Pro Gln Pro Pro Val Asn
                500                     505

GCT GGC GGC GGC TCT GGT GGT GGT TCT GGT GGC GGC TCT 1647
Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
    510                     515                     520
```

FIG. 11F

```
GAG GGT GGT GGC TCT GAG GGT GGC GGT TCT GAG GGT GGC 1686
Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly
            525                 530

GGC TCT GAG GGA GGC GGT TCC GGT GGT GGC TCT GGT TCC 1725
Gly Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser
535                 540                 545

GGT GAT TTT GAT TAT GAA AAG ATG GCA AAC GCT AAT AAG 1764
Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys
        550                 555                 560

GGG GCT ATG ACC GAA AAT GCC GAT GAA AAC GCG CTA CAG 1803
Gly Ala Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln
                565                 570

TCT GAC GCT AAA GGC AAA CTT GAT TCT GTC GCT ACT GAT 1842
Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr Asp
    575                 580                 585

TAC GGT GCT GCT ATC GAT GGT TTC ATT GGT GAC GTT TCC 1881
Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser
            590                 595

GGC CTT GCT AAT GGT AAT GGT GCT ACT GGT GAT TTT GCT 1920
Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala
600                 605                 610
```

FIG. 11G

```
GGC TCT AAT TCC CAA ATG GCT CAA GTC GGT GAC GGT GAT 1959
Gly Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp
        615                 620                 625

AAT TCA CCT TTA ATG AAT AAT TTC CGT CAA TAT TTA CCT 1998
Asn Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro
                630                 635

TCC CTC CCT CAA TCG GTT GAA TGT CGC CCT TTT GTC TTT 2037
Ser Leu Pro Gln Ser Val Glu Cys Arg Pro Phe Val Phe
    640                 645                 650

AGC GCT GGT AAA CCA TAT GAA TTT TCT ATT GAT TGT GAC 2076
Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp
            655                 660

AAA ATA AAC TTA TTC CGT GGT GTC TTT GCG TTT CTT TTA 2115
Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu
665                 670                 675

TAT GTT GCC ACC TTT ATG TAT GTA TTT TCT ACG TTT GCT 2154
Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala
        680                 685                 690

AAC ATA CTG CGT AAT AAG GAG TCT 2178
Asn Ile Leu Arg Asn Lys Glu Ser
                695         698
```

FIG. 11H

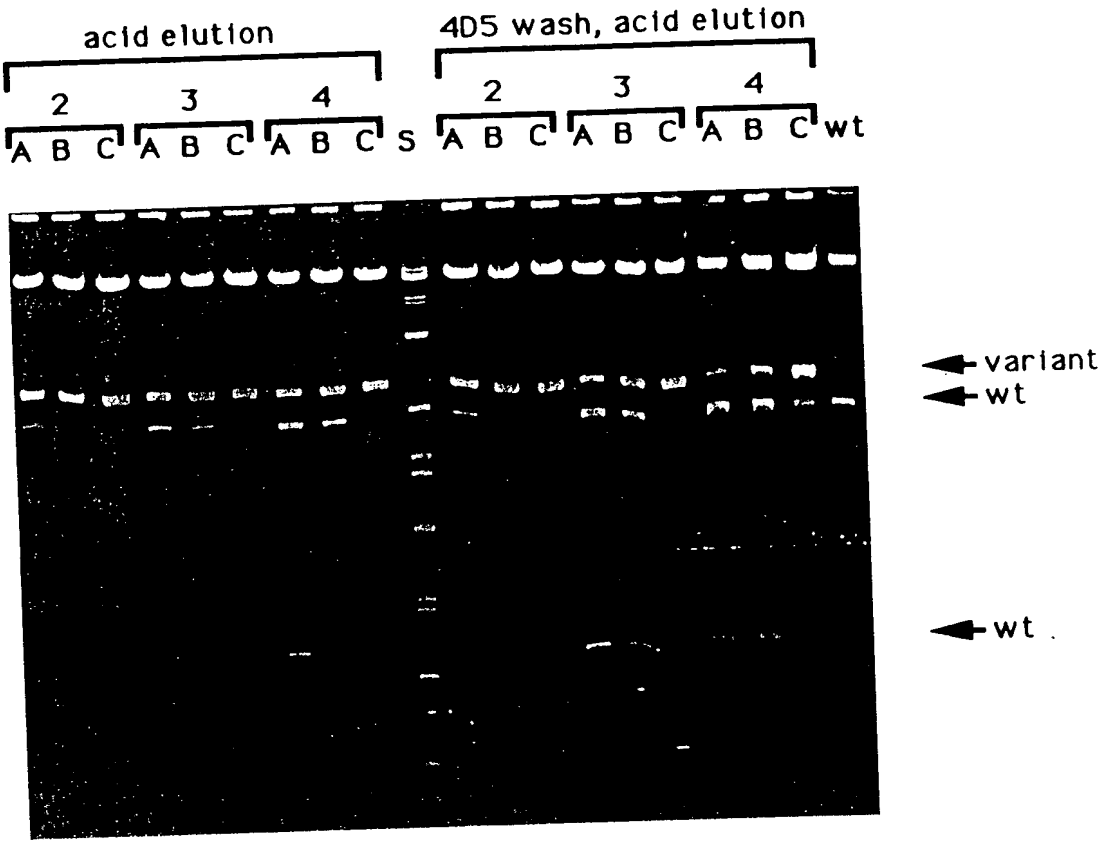

acid elution
4D5 wash, acid elution
2
3
4
2
3
4
A B C A B C A B C S A B C A B C A B C wt
variant
wt
wt

FIG. 12

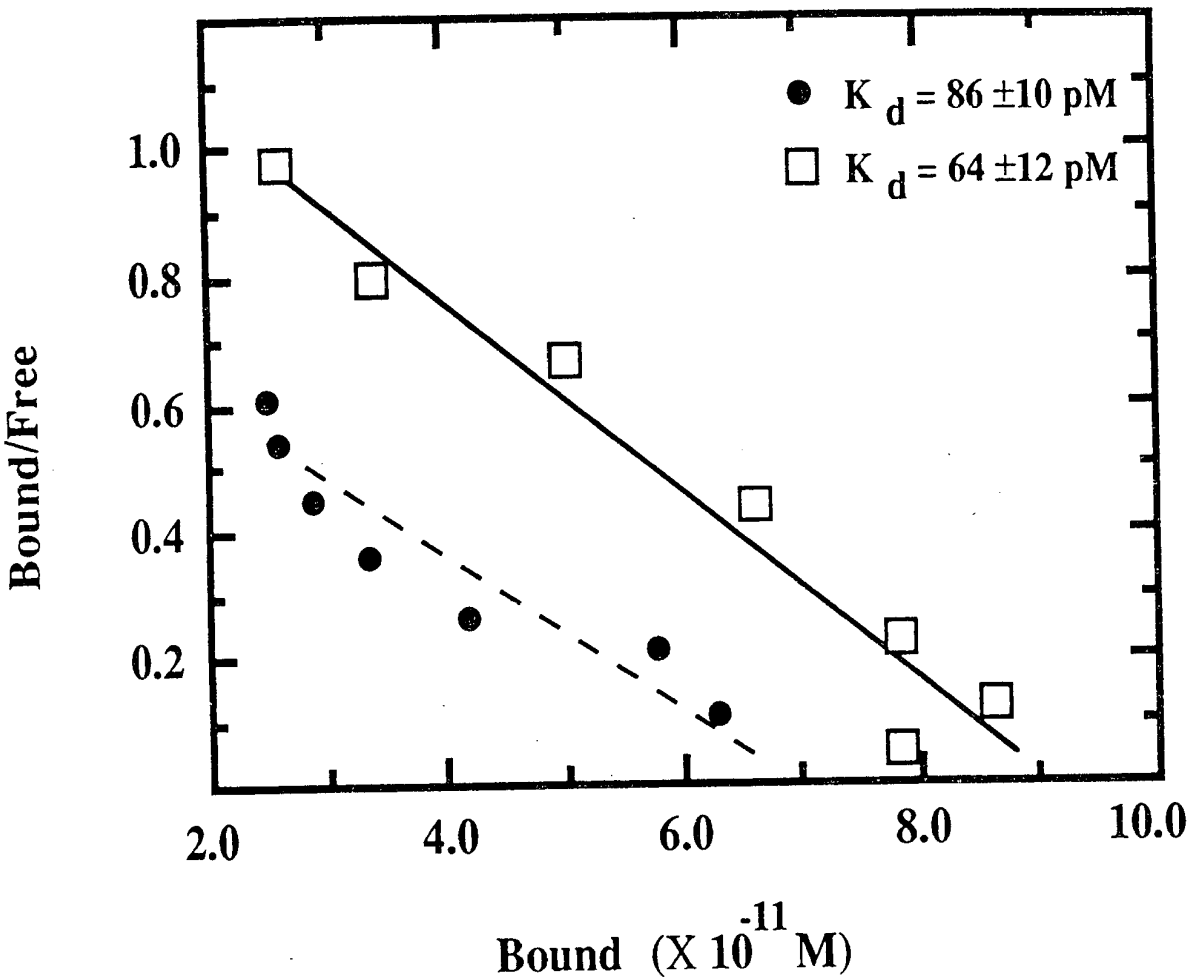


FIG. 13